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RAPID DIAGNOSIS OF THE HERPETIC VARIETY OF KAPOSI'S VARICELLIFORM ERUPTION BY TISSUE CULTURE METHODS*

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IN 1887 KAPOSI described the skin eruption that bears his name, and the following description is taken from the English translation of his textbook:[9] "A very alarming complication of eczema larvale infantum, which has come under my observation in several cases, is an acute outbreak of numerous vesicles, partly scattered, partly arranged in groups. The vesicles are as large as a lentil, filled with clear serum, and the majority are umbilicated. They look like varicella vesicles, but undoubtedly do not belong to this class. The integument which has been attacked in this manner now appears still more swollen, even tense. The little patients have fever (40° C. or more) and are very restless. The vesicles develop very acutely (sometimes overnight), in large numbers, and often continue to appear, in successive crops, for three or four days or even a week. Those which appeared first undergo desiccation, rupture and expose the corium, or they become encrusted and fall off. The largest number of these varicella-like vesicles are found upon the already eczematous skin, but smaller groups appear upon previously intact skin of the neighbourhood, upon the forehead, ears, neck, and even the shoulders and arms."

This eruption is now being increasingly recognized, both in children and adults, as a serious complication of atopic eczema, and the viruses of herpes simplex and vaccinia have been isolated from cases.[16] There may be other causes for the syndrome; for example, one case has been described following an attack of herpes zoster.[10] Now that facilities for the study of viruses are becoming more readily available, the causal agent can usually be promptly identified. When the causal virus is recognized, it is preferable to employ the appropriate specific term, eczema herpeticum or eczema vaccinatum.

The purpose of this paper is to report two cases of eczema herpeticum in which the etiological diagnosis was made within 24 hours of receipt of specimens by the isolation of herpes simplex virus in tissue cultures. The danger of the spread of infection to contacts is emphasized, as the second patient was a nurse in attendance on the first patient.

CASE HISTORIES

CASE 1

The first patient, S.M., aged 20, was admitted to the Toronto Western Hospital on February 14, 1955, for treatment of a generalized atopic eczema which had been resistant to treatment for several months. Three weeks before admission, she had a generalized, somewhat painful lymph node enlargement. On the fourth day after admission (Feb. 18) the patient developed a vesicular lesion on the left side of the upper lip, which in a day or so became vesiculo-pustular and spread to involve the upper cheeks and nose. Culture yielded a heavy growth of *Staphylococcus pyogenes*, sensitive only to erythromycin. These lesions, which were clinically herpetic, remained localized, and the patient's temperature normal, until the ninth day after admission, when the eruption spread very rapidly over the body, being particularly severe on the face, neck, palms, and soles (Fig. 1). On this day (Feb. 23) the temperature rose

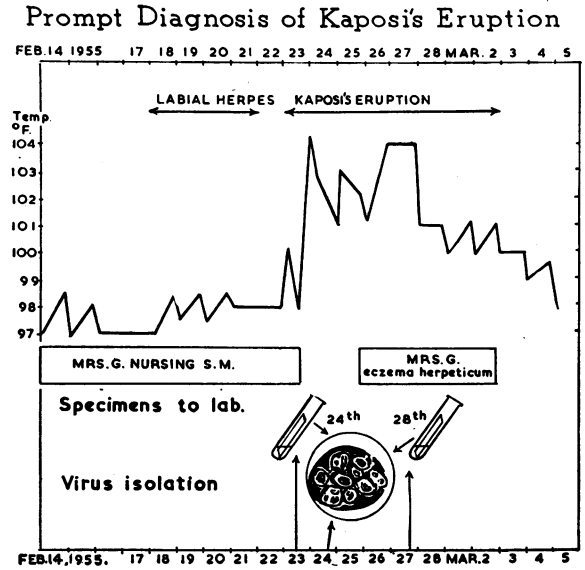

Fig. 1.—Temperature chart of Case 1 (S.M.) illustrating the time relationship of events in the two cases.

to 104° F. (rectal); she became seriously ill, and was very restless with a marked intolerance to pain. She also developed lesions in the mouth; an oliguria, previously of mild degree, became more severe. On general physical examination, only a mild bronchial irritation was found.

Specimens of blood, vesicle fluid and throat washings were taken for virus study. At this time, combined antibiotic therapy with streptomycin and erythromycin was instituted to control secondary infection. She was also given 12.5 c.c. of gamma globulin; this inoculation was followed by a prompt but brief fall in temperature, and improvement in her general condition. During the next five days, the patient was extremely restless and ill, but she developed no other signs of systemic involvement. The skin eruption became more pronounced.

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On the morning of March 2, her temperature began to fall and over the next five days returned to normal. At the same time her general condition improved, and her restlessness and intolerance to pain decreased. At the end of 10 days after the onset of the Kaposi eruption, antibiotic therapy was discontinued. At this time, her skin lesions, which had been vesicular and had spread over the entire body, began to recede. During this 10-day period, local therapy consisted of potassium permanganate dressings and antibiotic ointments, which afforded a measure of relief; sedative drugs had little effect in controlling her excitability and restlessness.

By the 21st day, there was no evidence of the eruption apart from a persistent erythema of the affected areas. The atopic dermatitis had completely disappeared and the lymph node enlargement improved. However, one week later, the atopic dermatitis recurred to a severe degree, and further treatment was instituted. The course of the illness was complicated by a phenobarbital eruption and an external otitis.

CASE 2

The second patient, Mrs. G., was the nurse responsible for the care and treatment of the first patient, S.M. Approximately one year previously, she had suffered from a contact eczematous eruption of her hands, caused by soaps and sterilizing solutions. She was able to carry on her work, using protective measures, and the dermatitis only rarely recurred.

Mrs. G. performed the dressings for S.M. from the time of admission to the hospital until Feb. 23, the day when S.M. developed Kaposi's syndrome. Mrs. G.'s hand lesions during this period were healed, and there was no evidence of eczema except for some slight thickening of the skin. On Feb. 26, a vesiculo-pustular lesion developed on the third finger of her left hand. Culture yielded *Staphylococcus pyogenes*. Within three days, a typical herpetic eruption appeared on both hands, and subsided after 10 days. The eczema again broke out but subsided after the patient had had two weeks off duty.

Mrs. G. was treated with potassium permanganate dressings and antibiotic ointments. In addition, at the suggestion of Dr. A. J. Rhodes, ether was employed to combat the herpes virus. Although this appeared to dry up the vesicles, it irritated the hands markedly, and may have been partly responsible for the exacerbation of the contact dermatitis.

VIRUS STUDIES

Methods

TISSUE CULTURES.—Rabbit cornea fragment tissue cultures were prepared by the standard method used in this laboratory.[5] Trypsinized human kidney monolayer cultures were prepared by a modification of the method of Youngner.[23] HeLa cells were maintained and prepared for viral inoculation essentially as described by Syverton *et al.*[20] For detailed microscopic examination, cultures were grown on coverslips, and fixed and stained with hæmatoxylin and eosin as described by Doane *et al.*[5]

Specimens.—Throat washings and swabbings from the skin lesions were placed directly into Medium 199,[13] containing 500 units of penicillin and 250 μg. of streptomycin per ml. This material was inoculated into tissue cultures. Acute and convalescent phase sera were obtained for serological study.

Viruses. For comparative purposes, a stock strain of herpes simplex virus isolated in Toronto (H51) was used in neutralization tests.

Virus titrations

Serial tenfold dilutions of virus were made in Medium 199, and 0.1 ml. amounts of each dilution were inoculated into five cultures. These titrations were performed in either human kidney or HeLa cell cultures; the results were read after one week by the observation of the cytopathogenic effect under the low power of the microscope. The 50% endpoint of infectivity was calculated by the Kärber method, and expressed in terms of the cytopathogenic dose (CPD_{50}).

SERUM NEUTRALIZATION TESTS

One hundred CPD_{50} of virus were added to serial fivefold or tenfold dilutions of serum. The virus and serum mixtures were allowed to stand for one hour at room temperature, and 0.1 ml. amounts were inoculated into five cultures of human kidney or HeLa cells at each dilution. The results were read after one week and the titre of the serum was expressed in terms of the final dilution producing inhibition of cytopathogenic effect in 50% of cultures.

RESULTS

Vesicle fluid, throat washings and blood obtained from S.M. on February 23 were inoculated into coverslip cultures of rabbit cornea the same evening. The next morning, the cultures inoculated with vesicle fluid were fixed and stained. Characteristic nuclear inclusion bodies of herpes simplex were present, thus enabling a provisional diagnosis of eczema herpeticum to be made within 24 hours of receipt of specimen. No virus was isolated from the throat washings or blood, despite several attempts in cultures of rabbit cornea, HeLa cells, and human kidney.

Vesicle fluid obtained from Mrs. G. on February 27 was inoculated into human kidney cultures because cultures of rabbit cornea were not available. Here also a presumptive diagnosis of herpetic infection was made within 24 hours, based on the finding of typical intranuclear inclusion bodies.

Subsequently, the cytopathogenic effect of the viruses isolated from both patients was shown

to be inhibited by an antiserum prepared by inoculation of rabbits with the stock H51 strain of herpes simplex.

Examination of acute and convalescent phase samples of serum from both patients showed a rise in neutralizing antibody to herpes simplex virus during the progress of infection (Table I).

TABLE I.

HERPES VIRUS NEUTRALIZING ANTIBODY LEVELS IN ECZEMA HERPETICUM

Patient	*Onset of Illness*	*Sera collected on*		*50% neutralizing titre**	
		Acute	*Convalescent*	*Acute*	*Convalescent*
S. M.	Feb. 23/55	Feb. 23/55	Mar. 14/55	$10^{-2.1}$	$10^{-3.1}$
Mrs. G.	Feb. 26/55	Feb. 27/55	Mar. 23/55	$10^{-0.6}$	$10^{-1.7}$

*H51 strain of herpes simplex used; similar titres obtained with sera of S.M. tested against homologous (S.M.) strain; titres given are in terms of final dilution of serum.

Since S.M. had a history of recurrent labial herpes, it is not possible to decide whether the initial antibody titre ($10^{-2.1}$) was due to previous infection, or was a response to the current infection. Mrs. G. developed antibodies during the course of the disease, as occurs in primary infections.

Human gamma globulin prepared from pooled adult blood in the Connaught Medical Research Laboratories, University of Toronto, was also examined and, as shown in Table II, it was found to have a high titre of antibodies to the stock herpes strain and to the herpes virus isolated from S.M.

TABLE II.

HERPETIC ANTIBODY CONTENT OF GAMMA GLOBULIN (CONNAUGHT LABORATORIES)

Source of herpes virus used in test	*50% neutralizing titre of gamma globulin**
H51 (stock strain)	$10^{-2.6}$
S.M.	$10^{-2.3}$

*In terms of final dilution of gamma globulin solution (16%).

DISCUSSION

Clinically, S.M. had a typical case of eczema herpeticum and conformed to the classical description of the severe form given by Kaposi.[9] Subsequent authors have referred to enlarged and painful lymph nodes as a prominent feature, but S.M. had such enlargement three weeks before the onset, so that this must have been related to the atopic dermatitis.

Mrs. G. had a localized infection of her hands, which were subject to contact dermatitis. Such milder cases are being increasingly recognized with the spread of virus diagnostic facilities.[8]

An etiological diagnosis can now be made promptly by the virus laboratory in most cases of Kaposi's syndrome. Herpes simplex and vaccinia viruses have been isolated from such cases,[16] and infection with these two viruses can be readily diagnosed by direct microscopical examination of scrapings or by isolation of the virus in tissue cultures. Microscopical examination of stained scrapings from lesions affords the most rapid diagnosis.[2] Giant cells with basophilic or acidophilic nuclear inclusions (Tzanck cells) are seen in scrapings from herpes simplex, but not from vaccinia. Elementary bodies are readily seen in suitable stained preparations from vaccinia, but are rare in herpes simplex.

Isolation of the causal virus is a much more sensitive method of diagnosis, and with the recent advances in the application of tissue cultures for virus study, this is within the scope of smaller laboratories. Rabbit cornea, human kidney, and HeLa cell cultures are all susceptible to herpes simplex and vaccinia. However, the cellular lesions are quite different; herpes simplex produces nuclear inclusions and giant cells, whereas vaccinia produces cytoplasmic inclusions (Guarnieri bodies) and no giant cells. A neutralization test with herpetic or vaccinial antisera serves to confirm the identity of the virus isolated.

The scrapings from lesions of zoster or varicella are identical in appearance to those from herpes simplex.[2] In view of this, and the possibility that the virus of zoster may cause some cases of Kaposi's syndrome,[10] these viruses have to be considered in the differential diagnosis. Neither zoster nor varicella virus will grow in rabbit cornea tissue, and Weller[21] has only recently succeeded in propagating them in cultures of human tissue. Zoster and varicella viruses produce similar focal lesions with giant cells and nuclear inclusion bodies; the viruses are indistinguishable serologically.[22] These viruses differ from others in that cell-free material from infected tissue cultures cannot be used to transmit the infection in series.[21]

Vaccinia cannot be distinguished from variola by the methods so far described. McKenzie,[11] during the 1942 outbreak of smallpox in Glas-

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TABLE III.

KAPOSI'S VARICELLIFORM ERUPTION: IDENTIFICATION OF CAUSAL VIRUS

	Smears or scrapings of lesions		*Growth in tissue culture of*			*Other tests*	
Viruses	*Elementary bodies*	*Giant cells*	*Rabbit cornea*	*HeLa cells*	*Human kidney*	*Inoculation of chorioallantoic membrane*	*Serology*
Herpes simplex	Scanty	Numerous with nuclear inclusions	Rapid formation of giant cells with nuclear inclusions	Similar to rabbit cornea, growth less rapid	Similar to rabbit cornea	Small discrete lesions	Neutralized by herpetic antisera
Varicella and zoster	Scanty	Numerous with nuclear inclusions	No growth	No growth	Foci of giant cells with nuclear inclusions. Viruses not transmissible without cells	No growth	Varicella and zoster not distinguishable
Vaccinia	Numerous	Absent	Grows well with cytoplasmic inclusions	Grows well	Grows well	Diffuse hæmorrhagic necrotic lesions	Vaccinia and variola not distinguishable
Variola	Numerous	Absent	Not known	Not known	Not known	Discrete white lesions	Vaccinia and variola not distinguishable

gow, noted 10 cases of Kaposi's eruption representing many times the normal incidence. Crusts from two of the cases gave positive serological results for vaccinia, but in view of the outbreak of smallpox, preventive measures appropriate to the more severe disease had to be taken. The laboratory diagnosis of smallpox and the distinction between variola and vaccinia viruses are discussed by Downie and MacDonald.[7] Vaccinia virus produces diffuse hæmorrhagic necrotic lesions on the chorioallantoic membrane of the developing chick embryo, and grows well on rabbit skin, whereas variola virus produces discrete white lesions on the chorioallantoic membrane, and grows poorly on rabbit skin.

Criteria for distinguishing between these viruses are summarized in Table III. Since the majority of cases are due to vaccinia or herpes simplex, the examination of scrapings and inoculation of one of the three varieties of tissue cultures mentioned will suffice for routine purposes. Tissue cultures of rabbit cornea are at least as sensitive as classical methods of isolating herpes simplex and very much more rapid.[5] This rapidity is particularly valuable in diagnostic work and is well illustrated by our cases. Tissue cultures also facilitate accurate quantitative serological titrations.

Mrs. G. probably experienced a primary infection, since she had a very low level of antibodies in the acute phase serum and a clear-cut rise in titre in the convalescent specimen (Table I). The infection remained localized to the susceptible cells of the skin of her hands which were infected by direct contact with S.M.

The pathogenesis of the infection of S.M. is less obvious. She gave a history of recurrent labial herpes and her most recent attack started five days before the generalized eruption, and must therefore have been the source of the virus. The onset was so abrupt as to suggest blood-borne dissemination of virus (Fig. 1). The failure to isolate virus from the blood may have been due to sampling after the termination of the viræmic phase. Similarly the high and rising antibody titre might be explained as an accelerated secondary type response in a person whose antibody mechanism had been sensitized by previous exposure to the virus. There is good evidence for viræmia in some cases of primary herpes,[8, 15] and circulating antibody appears in the blood about 5-6 days after a primary infection.[18] Some observations by Downie and McCarthy[6] on the pathogenesis of smallpox are relevant in this connection. In smallpox the virus spreads to the skin by the

blood stream, yet in patients who survive, it can rarely be recovered from the blood after the appearance of the rash. Also patients who develop smallpox, despite previous vaccination, produce antibodies very rapidly so that the latter are present three days after the onset in most cases, about two days before their appearance in unvaccinated cases. The appearance of antibody is associated with clinical improvement.

Alternatively, virus may have spread directly over the skin from the labial focus. S.M. was suffering from a very acute weeping eczema treated with moist dressings. The rash was very irritating, and scratching, especially at night, was unavoidable. Conditions were, therefore, ideally suited to the surface spread of infection. The antibody titre during recurrent episodes of herpetic infection does not as a rule alter from the level established after primary infection.[18] Therefore, in view of S.M.'s history of recurrent herpes, the antibody titre of $10^{-2.1}$ observed on February 23 was probably present on February 18 also. The titre is very similar to that observed in Mrs. G. during convalescence and falls within the range found by Scott *et al.*[18] It is reasonable to suppose that this antibody might not protect skin cells against direct infection, but it should preclude dissemination of virus by the blood stream. The presence of antibody certainly offers a reasonable explanation for the failure to isolate virus from the blood. The tenfold rise in antibody titre observed in convalescence was presumably due to the widespread infection and massive absorption of virus antigen.

Most cases of eczema herpeticum give a history of recent exposure to herpetic infection.[1] They either infect themselves like S.M. or are exposed to a source of infection like Mrs. G. Several epidemics in skin wards have been described.[4, 12] While the majority of cases occur as a complication of atopic eczema, any area of damaged skin is susceptible to infection.[1, 19] Cases of atopic eczema should therefore be protected from all sources of infection.

Several cases of recurrent eczema herpeticum have been reported.[3, 17, 19] It is not clear whether these recurrences are due to reinfection of the cells of the skin from an isolated focus of latent infection or to a recrudescence of a widespread latent infection. The case described by Boake *et al.*[3] had several recurrences in an area of skin subject to atopic dermatitis. Mild stimuli such as a fever provoked only labial herpes, but increasingly severe stimuli led to the involvement of more skin areas, which suggests that more widely seeded, latent virus was activated by the severe provoking stimuli. Patients with eczema herpeticum shed large amounts of virus into their environment and should be nursed in separate cubicles with full barrier precautions. Secondary bacterial infection, usually with *Staphylococcus pyogenes*, is almost invariable, and formerly added greatly to the severity of the cases. Such bacterial infection should be treated with the appropriate antibiotic, or combination of antibiotics, based upon sensitivity tests with the organism isolated from the patient.

Ether is known to destroy the virus *in vitro* and might be expected to control the surface spread. It appeared to exert a beneficial effect on the herpetic infection, but may have been responsible for the aggravation of the eczema, when given to Mrs. G. Subsequently, one of us (H.C.H.) has used ether for the local treatment of several herpetic eruptions with encouraging results.

Gamma globulin has a high titre of herpes simplex neutralizing antibodies (Table II). In view of the possibility of viræmia[8, 15] it is indicated in the treatment of severe primary infections. It cannot be expected to influence favourably a case like S.M., where circulating antibody is already present. Treatment with cortisone is contraindicated, because it has been shown to aggravate experimental herpetic keratitis in the rabbit.[14]

SUMMARY

1. Two cases of eczema herpeticum are reported.

2. Herpes simplex virus was isolated by tissue culture methods from both cases within 24 hours of receipt of specimens.

3. A rise in titre of antibodies to herpes simplex was demonstrated.

4. The diagnosis, pathogenesis and treatment of the condition are discussed.

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EFFECT OF NIACIN AND NICOTINAMIDE ON LEUKOCYTES AND SOME URINARY CONSTITUENTS*

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NICOTINIC ACID and its amide have been used for the treatment of many mental disorders uncomplicated by pellagra or even mild subclinical deficiencies.[1-8] For the past three years we have used this vitamin in large dosages (3-10 g. per day orally) for the treatment of acute schizophrenia to bring about remission and to prevent relapse[9] and as an adjunct to anticonvulsant therapy for epilepsy.[10] Three grams per day has been given for up to three years with no toxic effects. It is therefore of great interest to determine the action of these large quantities on body physiology.

Recently nicotinic acid has been found to counteract many of the psychological and physiological reactions of lysergic acid diethylamide,[11] to lower blood cholesterol,[12] to counteract the action of adrenochrome on the electroencephalogram of epileptics, and to have anticonvulsant properties.[13]

The changes in blood leukocytes and in some urine constituents are reported here.

METHOD

Nicotinic acid or its amide was administered orally to two groups of healthy volunteers: (1) a group of 31 subjects ranging in age from 18 to 27 (mean 21.8) including three women, and (2) a group of 11 subjects ranging in age from 21 to 41 (mean 30) including seven women. The latter group comprised the two-gram nicotinic acid group. Samples of blood (intravenous) and urine were taken just before the administration of the vitamin (9.30 a.m.) and two, four and 24 hours after the first dose. The two- and four-hour values were all determined after the initial dose (1 or 2 g. as indicated), but the 24-hour value was determined after four doses, i.e. the second dose after the four-hour sample, the third dose at bedtime and the fourth dose the next morning, two hours before the final sampling.

The blood was examined for leukocytes, and eosinophils were counted. The urine was examined for sodium and potassium (flame photometer), for pH (using pH electrometer), for uric acid and creatinine and for glycine by quantitative paper chromatography.[14]

RESULTS

The changes induced in leukocytes by the acid and amide are shown in Table I.

TABLE I.

THE EFFECT OF NICOTINIC ACID AND NICOTINAMIDE UPON LEUKOCYTES IN BLOOD

Treatment		*No.*	*Initial counts*	*Percentage change produced at 2 hours*	*4 hours*	*24 hours*
			Eosinophils			
Acid	2 g.	11	217	−57	not done	not done
	1 g.	11	88	−30	−35	20
Amide	2 g.	10	88	0	−40	15
	1 g.	10	121	8	−20	8
			Neutrophils			
Acid	2 g.	11	4,155	115	not done	not done
	1 g.	11	4,125	60	162	15
Amide	2 g.	10	4,195	0	44	0
	1 g.	10	3,934	29	28	10
			Lymphocytes			
Acid	2 g.	11	2,486	−20	not done	not done
	1 g.	11	2,461	−5	15	2
Amide	2 g.	10	2,185	18	25	33
	1 g.	10	2,149	43	20	46

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